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ORIGIN OF THE CENTRAL AND OSTIOLAR CAVITIES IN PYCNIDIA OF CERTAIN FUNGOUS PARASITES OF FRUITS[1]

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SCOPE AND PURPOSE OF THE INVESTIGATION

An investigation of fungi associated with rotting strawberries and dewberries during shipment and marketing has afforded an opportunity for study of the development of the pycnidial form of several species of fungi, from which it appears that in these forms there are three principal stages or periods of growth. The first is one of rapid cell multiplication whereby a certain amount of fundamental or undifferentiated tissue is formed with which to begin the construction of the fruit body. The second or intermediate stage includes those activities leading to the formation of the central cavity, the organization of the wall, the delimitation of sporogenous tissue, and, in many forms, the construction of the ostiole. Previous study has largely been confined to the third stage, which includes spore production and other developments which characterize the mature pycnidium. The methods by which the plectenchymatous primordium is formed are also fairly well understood, but just how the characteristic pycnidium is evolved out of the mass of undifferentiated tissue has not been clearly explained. The intermediate stages in the growth of phycnidia of several species have been followed by the writer, and the results of the studies on three of these are reported in this paper.

Bauke's contribution (*2*)[2] is noteworthy because of the clearness with which he describes the two different methods by which pycnidia originate; and on this point, which was his main contribution, his work has been repeatedly confirmed. From the very nature of the subject of his investigation, the method which he employed was not adequate to give the best results. He found it difficult to learn what takes place in the pycnidial "knot" when the cavity is being formed and the sporiferous layer organized, as he was compelled to study the fungus as it grew on slides. In the pycnidium of *Cucurbitaria elongata* a cell at the center becomes divided into a few radially arranged pyramidal cells with their apices together at the center. These are the sporophores or their mother cells. They divide again in various directions or at once cut off spores. By the gelatinization of their walls, the sporophores are separated from each other to form the cavity, which is further increased by the swelling of the mucilage derived from the walls of the spores. In the pycnidium of a Diplodia on Cornus there is at the end of the first period of growth a

[1] Accepted for publication Oct. 22, 1921.
[2] Reference is made by number (italic) to "Literature cited," p. 758–759.

pseudoparenchymatous structure of thin-walled cells bordered by a cortical region. Now at various points in the central portion there appear elliptical areas marked by inwardly growing hyphæ. There are a number of these regions of growth corresponding to the number of locules which will appear in the young pycnidium; some of them may become united as the phycnidium ages. According to Bauke it is the head-on thrust of these young sporophores against each other and the pressure due to the gelatinization of their walls that accounts for cavity formation by forcing the sporogenous layer farther and farther out, crushing the tissue between it and the cortex so that the protoplasm of this crushed tissue can be used in nourishing the spores.

De Bary (*3*), without referring to Bauke's account, describes the development of the pycnidium of a species of Pleospora and says that the central cavity is due to the failure of the cells at the center of the pycnidial primordium to keep pace in their growth with those in the periphery so that the cells at the center are torn asunder. Such a cavity would be schizogenetic.

Baccarini (*5, p. 69*) describes the formation of a cavity in *Sphaeropsis malorum* and adds a further note on the development of pycnidia in general (*6, p. 150–151*). Certain cells at the center of the pseudoparenchyma can be distinguished from the rest by their abundance of oil and glycogen. Their walls become transformed into mucilage which, absorbing water, swells to form a central, oval, or spherical cavity lined with sporophore fundaments. This sporiferous layer plays an important part in the further development of the pycnidium. Baccarini does not say that the entire contents of the cells at the center disappear as the cavity is formed. His account is very similar to that given by Bauke, adding nothing except to state (*6, p. 150*) that lysigenetic and schizogenetic factors enter into the processes, depending upon the quantity of tissue present at the center of the primordium and on the rapidity with which it is reabsorbed.

Reddick (*8*) studied the development of the pycnidium of blackrot of the grape. He states that the activity at the center of the "gnarl" is evidenced by the more deeply staining contents of the cells. He finds that a number of hyphae grow inwardly and assumes that these are the young sporophores. He realizes that the cavity later increases in size, and he believes that it does so in some way at the expense of the pseudoparenchyma. In his description of the origin and the development of the perithecia the nature of the "sclerotium" is discussed. He believes that such a body develops into a perithecium and gives it the name pycnosclerotium.

Hesler (*10*) describes briefly the development of the pycnidium of *Sphaeropsis malorum*. His account is similar to that given by Baccarini, to which he refers. Hesler, however, states without further comment or figures that the cavity is formed by the breaking down of cells and that the ostiole is formed in the same way. He is of the opinion that the dome-shaped tissue (often shown in figures of this pycnidium) from which the sporophores arise is due to irregularities in the cavity which is tending to become multilocular.

In order to best illustrate the relative importance of the two factors, disorganization and growth inequalities, in cavity formation, the intermediate stages in three pycnidia will be described. The formation of the ostiole will be considered in connection with one of the forms.

BLACKROT FUNGUS OF DEWBERRY

There is a blackrot fungus of dewberries the pycnidia of which are very similar to those of *Phyllosticta paviae*, *P. labruscae*, and *P. solitaria*. Pycnidia frequently develop on berries from North Carolina found in the New York and Washington markets. It requires but a short time for the black rot to make its appearance if berries are placed in damp chambers. On account of the slowness with which the disease develops in transit and market it will probably not prove of much economic importance.

The fungus has not been connected with its perfect stage, but it may very well belong to *Physalospora carpogena* Atkinson, authentic specimens of which apparently are not in existence. The pycnidial stage has therefore been called by Shear *Phyllostictina carpogena*, and a formal description is to be published.

The fungus has been isolated, and pycnidia have been grown to maturity on agar media. Dewberries artificially infected with spores from these cultures have developed the typical blackrot. An abundance of material from natural sources and from these cultures has been available for study.

FORMATION OF THE CENTRAL CAVITY IN THE PYCNIDIUM

The "gnarl of hyphae" develops into a plectenchymatous body which has been called a "pycnosclerotium" (Pl. 1, A), such as has been noted and described many times by those who have studied the blackrot of the grape. The large outer cells appear dark in color with thick walls, but toward the center the cell walls are very thin. The central portion possesses sufficient food for future structural growth processes. While in this fungus, as well as in that of grape blackrot, it is undoubtedly true that similar bodies are immature spermogonia, or young perithecia, there can be no question that every pycnidium of both species passes through some such form in its development. If cavity formation for some very definite reason begins early, while the primordium is still plastic, then the resemblance to a "sclerotium" is not so marked. These bodies are frequently as large as mature pycnidia. If in such case there is little further enlargement, how may we account for the central cavity of the pycnidium? In the sections shown in Plate 2, A, B, and Plate 1, B, there is evidence of the beginning of disorganization of cells at the center. As soon as one or two cells disintegrate sufficiently, the surrounding ones push into the mucilaginous matter and in their turn begin to disorganize. It may be that degeneration is at first due to the head-on thrust of cells pushing inwardly; but, if one may judge by what follows, it is more likely that the breaking down process begins first.

The cells of the next succeeding layer to push into the small open space present the appearance of rather thick sporophores, which is misleading (Pl. 2, C, D; 1, C), since ordinarily these also become disorganized by degrees, and the cells below them push out in their turn. The wall layers are not as yet well organized. The conditions in this stage are shown in Figure 1, *a*. The cells lining the cavity clearly result from the budding or division of the cells of the original pseudoparenchyma. They do not show in this section very decided effects of pressure from within, such as Bauke (*2*) says results in crushing and flattening the inner-wall cells. Later, as the cells elongate or even divide tangentially, this

appearance is more exaggerated. From each of these cells two or three hyphae now branch out, each composed of one to several cells, the outermost being in various stages of disorganization. The nuclei are quite distinct; frequently there are two lying close together in each cell. So similarly do the cavities in pycnidia and spermogonia originate that one can not be certain into which such a structure will eventually develop. These protosporophores occasionally produce spores, since a few pycnospores sometimes are found in sections of such young pycnidia, just as spores are rarely budded off directly from the thin-walled cells at the

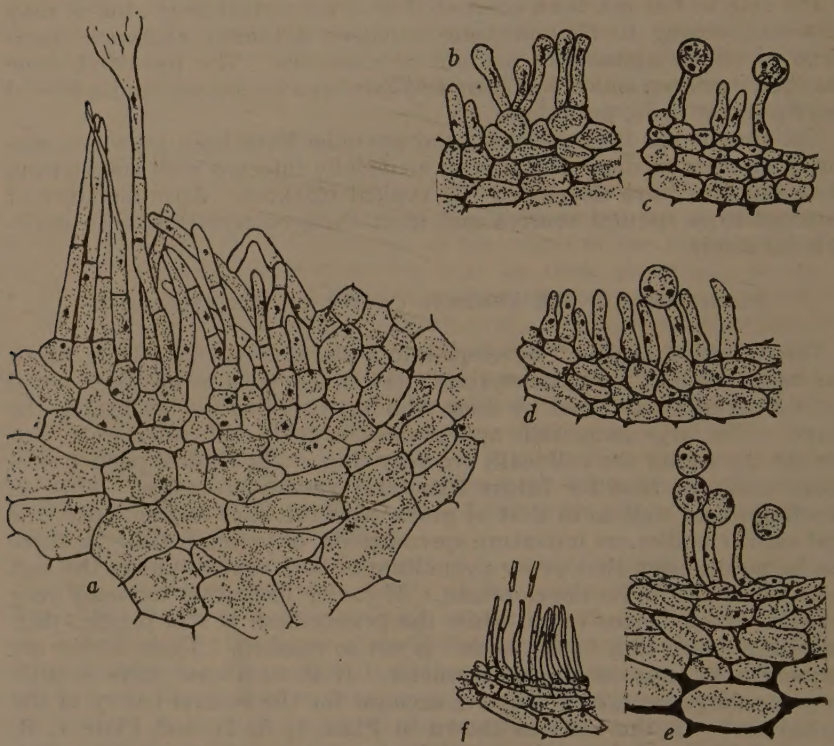

FIG. 1.—Development of sporophores: a, Hyphalike protosporophores growing out from cells of wall which is as yet not definitely organized, showing ends of hyphae undergoing disorganization; b, the first sporophores, sometimes several budding from a single cell, with ends of sporophores swelling in first stages of spore formation; c, sporogenous layer now definitely organized; d, young sporophores, each with a single nucleus; e, sporophores from a mature pycnidium, each spore binucleated; f, sperma tiophores from a mature spermogonium.
All drawings made with the aid of a camera lucida Zeiss No. 8 ocular, 3-mm. oil-immersion lens.

very beginning of cavity formation. This may account for the pycnospores sometimes found in spermogonia, to be noted later.

The ostiolar portion has not been developed in the particular pycnidium shown in Plate 1, C; central cavity formation began very early, while the primordium was still little "carbonized" and therefore in a plastic state. Sections often show a large central cavity filled with pieces of the inwardly growing hyphae in all stages of disintegration. New buds to form sporophores arise from the subadjacent cells, which as the result of radial and tangential divisions become much smaller than those which originally occupied this space in the pycnosclerotium. A still older stage is shown

in Plate I, D, where there are no traces of the hyphalike branches; but the big cavity is filled with the stainable disorganized remains, suggesting very strongly that much of the nutrient for spore formation is obtained from within. In more mature pycnidia the central and ostiolar cavities will contain many spores, but no stainable cell remains (Pl. 1, E).

Sporophores are less easily distinguished in mature pycnidia because they are used up more and more as the food supply becomes exhausted in spore formation. This was pointed out by Bauke (*2*). It would be difficult to prove in any case, but it is not improbable that the entire layer of fertile cells bearing the first functioning sporophores may disappear and new sporophores, one by one, grow out from the cells next in line. The cavity certainly increases in size, and the extent of the tissue lining it diminishes. Plate 1, D and E, shows an elliptical cavity in the necklike portion which serves as a temporary storage space for spores.

SPERMOGONIA OF THE FUNGUS

One finds statements regarding the transformation of spermogonia into pycnidia, or vice versa, or of either of these forms into perithecia. No one has given us the story of what happens to the pycnidia covering a particular grape placed out of doors throughout the winter. After a somewhat extended study of this fungus, the writer is still unable to distinguish positively the bodies out of which spermogonia or pycnidia will be formed. He suspects that each of these spore-producing bodies arises from its own peculiar primordium, notwithstanding the fact that it is not at all rare to find pycnospores in spermogonia.

As noted previously (p. 745), the perfect stage of *Phyllostictina carpogena* remains in doubt. Further evidence that it may be a Guignardia is that it has a spermogonium very much like that of *Guignardia bidwellii*.

Spermogonia and pycnidia may be found side by side on the dewberry, and were it not for their contents these fruit bodies could not be distinguished. In size, shape, and wall structure they may be identical. In many spermogonia there is found the same peculiar elliptical ostiolar cavity (Pl. 1, F) so characteristic of pycnidia of this species. The writer has studied much more extensively the formation of the cavity in spermogonia of *Guignardia bidwellii*. The processes appear to be the same in both species.

CAVITY FORMATION IN THE SPERMOGONIUM

Cavity formation begins rather in the apical portion of the primordium of fundamental tissue where by partial disorganization of cells way is made for growth of orientation by which chains of cells become directed toward the apex. This stage, once initiated, is carried on very rapidly. Protospermatiophores which are long and threadlike soon fill the central portion (Pl. 1, F), each hypha (?) consisting of a chain of rodlike cells surrounded by a mucilaginous sheath. These cells, freed from each other, could scarcely be distinguished from spermatia; and it is possible they are the first spermatia formed. Later, when definite spermatiophores (Fig. 1, f.) can be clearly made out, the cavity is devoid of such threadlike aggregations, and very distinct rod-shaped spermatia lie around in the cavity. This suggests that the structures shown in Plate 1, F and G, now break up and disorganize, leaving the mucilaginous products still in the cavity, and that definite spermatia will soon be formed.

There are eight pycnospores in the spermogonium, a section of which is shown in Plate 1, G. There is now no evidence of the origin of these spores, which were probably formed at an early stage in cavity developement; and the cells from which they were derived have now disappeared. The writer has not seen sporophores in those spermogonia of *Guignardia bidwellii*, which contain a few pycnospores.

It has been shown above that spermogonia and pycnidia are formed from bodies similar to what Reddick (*8*) calls "pycnosclerotia." In many species the tissue composing the "gnarled" primordium is not at all sclerotized. Pycnidia of *Schizoarme straminea* Shear, for example, arise out of a tissue that can not be distinguished from a stroma (Pl. 4, A). The very irregular tuberculate and massive forms taken by the "pycnosclerotia" of *Phyllostictina carpogena* in cultures on agar very frequently become arrested at this point, but so many have been found in the process of cavity and spore formation as to suggest that these structures are fundamentally sclerotized stromata, out of which, or in connection with which, fruit bodies are formed. Pycnosclerotia then, if we may put it that way, are not designed to be resting stages in the life cycle, not necessarily structures into which the fungus is forced (even if frequently compelled to remain there) by unfavorable environmental conditions. They are the result of a regular and natural course of events by which certain quantities of food and fungous tissue are brought together in preparation for spore formation. Having seen that in this Phyllostictina there is little actual crushing of tissue such as was thought by Bauke (*2*) to be the case, the relative importance of the two factors, disorganization and growth inequality, in cavity formation can be judged also by the little average difference in size between the "pycnosclerotia" and mature pycnidia. Allowing for cases such as were noted previously in which cavity formation begins very early, the central cavity in this species is ordinarily, then, largely lysigenetic. We may next turn to a pycnidium of an entirely different type.

ORIGIN OF THE CENTRAL CAVITY IN THE PYCNIDIUM OF THE STRAWBERRY-ROT FUNGUS

Associated also with the rots of dewberries and strawberries is the fungus, *Sclerotiopsis concava* (Desm.) Shear and Dodge, and its conidial stage is very common on other plants (*12*). The pycnidium is large, shield shaped, and without ostiole. The big spore cavity is surrounded by a heavy brown wall several cells in thickness and at maturity is densely packed with spores (*12*, Pl. 5, Fig. 17).

The intermediate stages in development are best studied from sections of material grown on strawberries or on infected leaves placed in damp chambers. In the younger primordia, hyphae invade the epidermal cells, bursting through the side walls and lifting the cuticularized layers, forming a broad, compact, mound-shaped tissue (Pl. 3, A), most of the outer portion of which consists of hyphae spreading outward from a broad base (Pl. 3, B). The cells at the ends of these hyphae after a succession of divisions become elongated peripherally. Now along a line 5 or 10 cells from the outside border a narrow zone of degeneration can be distinguished; cells are losing their contents and elongating as though under strain (Pl. 3, C). This disorganization occurs in intercalary cells shown at the right in the figure. The line of rupture is not necessarily so evenly curved that it follows the contour of the outer wall;

it is more often very irregular, dipping down here and there rather deeply (Pl. 3, D). Superficially the pictures presented certainly suggest that the rupture is caused by forces from within, either that the lower two-thirds of tissue has shrunken while the outer third designed as the future wall has remained rigid or that the latter part has been raised bodily by addition of new cells at the ends of the arch, the rest of the tissue remaining dormant. But the early stages of these large, dome-shaped pycnidia develop in nature beneath the tough and tightly stretched cuticle, for example, of canes of *Rubus* spp., where if the rupture were caused in the manner just suggested, there would occur more or less buckling of the outer wall under such unequal strains as must exist. At such a stage (Pl. 3, D) no buckling occurs, which means that the wall must be supported by the pressure from below, furnished by disorganization of the intercalary cells, as indicated. Mature pycnidia on leaves in dry weather are found collapsed, indicating that the thick arched wall in itself is not strong enough to prevent buckling after the pressure has been withdrawn by the shrinkage of the mucilaginous substance within. A concave pycnidium usually indicates that it will be found full of spores. Figures D and E are from a section of the same pycnidium at slightly different planes. Figure E shows somewhat better that the break occurs in radially growing hyphae, but Figure D suggests that disorganization accompanied by the swelling of cells is responsible for the rupture of the tissue. The five or six large bodies mixed in with the tissue just pulling apart (at the center above) are the remains of what just previously were very minute cells of the parallel hyphae and which have now swollen to many times their original size. Many such cells are visible in the section shown at F and also at H, which is still more highly magnified. Above are large masses of small intercalary cells detached from the hyphae which bore them and are now caught between two regions of disorganization. In the section shown at G, the sporophores are fully formed along the entire base; but the mass above these consists only of rows of disorganizing cells, above which is a clear substance, difficult to stain. In this condition the structure might be mistaken for a mature pycnidium nearly filled with spores. Compare this with the section shown at I, which is from a very small pycnidium in which the first spores have just been formed. The spreading, or nearly parallel, rows of cells forming the upper wall are still distinct. The cavity may now enlarge to some extent, and there is little question that growth at the border accounts for some increase in its size. It is noteworthy that there is always more tissue below the line of rupture in a young pycnidium than there is below the true sporophores in a mature structure (Pl. 3, C, D, and I). The cells that are first forced apart in the destruction of tissue and that remain below are homologues of the sporophores, but as noted they provide the material which by swelling forces the wall farther out, incidentally later furnishing food for the development of spores.

It has been shown that the rupture in the tissue is initiated by the disorganization of certain intercalary cells in oriented hyphae and that this occurs not at a period of shrinkage but while expansion is still going on. Pressure is provided by the swelling of the cells torn loose from the wall above or isolated from the vertically growing hyphae below. This force maintains the even curve of the wall as it lifts the resistant portions

of the epidermis above, and marginal growth contributes to the final enlargement of the cavity. We see, then, that the cavity in *Sclerotiopsis concava* is about equally lysigenetic and schizogenetic, and in this respect represents a type intermediate between *Phyllostictina carpogena*, noted previously, and *Schizoparme straminea*, which will now be considered.

SCHIZOPARME STRAMINEA, A NEW STRAWBERRY DISEASE

Pycnidia of a fungus heretofore unreported on strawberry have been found on this fruit from Norfolk, Va., and in the markets of New York and Washington each spring since 1918. The fungus is not abundant, and it does not cause a destructive rot of the berry. The pycnidia are thickly scattered over the surface bursting through the epidermis at the time the spores are mature (Pl. 5, A). The fungus is distinguished from others on the strawberry by the crown of light-colored tissue which surmounts the pycnidium and surrounds the ostiole. The same species also occurs on dead leaves of strawberry and several other plants. In July, 1920, perithecia were found on strawberry leaves along with these pycnidia, and the connection between the two forms was proved by growth in pure cultures. Pycnidia and perithecia are illustrated in Plates 4, 5 and 6. A formal description of the fungus is to be published by Doctor Shear in an early number of Mycologia.

CULTURE STUDIES

The unicellular pycnospores germinate with one or two germ tubes without showing septation previous to or during germination. On corn-meal or potato agar in Petri dishes the mycelium grows outward unevenly, so that the margin is much scalloped or lobed, (Pl. 5, B). Pycnidia are formed in concentric circles, the first maturing five or six days after inoculation. They are at first colorless and are surrounded by a loose weft of hyphae. Sclerosis begins at the base of the ostiole and spreads around the wall along the outer layer of cells, so that the pycnidium finally becomes nearly black.

Artificial infection of strawberries has been made to obtain material for study. The fungus is able to maintain possession except when species of Rhizopus gain entrance at an early stage.

In Petri-dish cultures there is a tendency toward the grouping of pycnidia, with an especially large one at the center (Pl. 5, D). They are held together by the tissue which crowns each one and which now viewed from above resembles a thin stroma; the fruit bodies are always separate in nature.

The ascospores are small and hyaline and thus are not readily located in poured plates, so that the following method was used to obtain pure cultures. Single ascocarps were crushed out in a drop of water on sterilized slides. The asci still containing spores float out in the water. Small drops of this water were transferred to a 4 per cent corn-meal agar in Petri dishes at marked places. As the drop spread out and was absorbed, single asci were located. The spores germinated in the ascus in a few hours, so that by transferring the entire ascus after germination one was able, very easily, to obtain large numbers of pure ascospore cultures. Over 100 single ascus cultures in test tubes and Petri dishes were made in this way, and in every case characteristic pycnidia were

formed in a few days. No perithecia have been seen in cultures either from ascospores or pycnospores.

FORMATION OF THE CENTRAL CAVITY IN THE PYCNIDIUM

The various stages are best studied in material obtained by artificial infection of strawberry and from cultures on agar media where the early stages are somewhat exaggerated and prolonged, so that the growth story can be followed and timed more easily than from pycnidia obtained on fruit and dead leaves. Sections of the pycnostroma show a rather homogeneous plectenchyma of thin-walled cells without any trace of sclerosis; consequently, as we should expect, the force due to the more rapid growth of the peripheral cells resulting in the rupture of inner cells comes most easily and naturally into play.

When the primordium has attained considerable size, forming a spherical or elliptical mass of tissue 50 to 100 microns in diameter (as it is found on fruit or on leaves in nature it is much less), it has stored in it a quantity of food readily available for the developments which are next to take place. The sporogenous tissue will be located on a dome-shaped structure placed at the bottom of the cavity similar to that of *Coniothyrium diplodiella* described by Istvanffi (7); the curve of the dome will correspond roughly with that of the wall above, with a spore cavity between the two tissues. The region in the plectenchyma where the cavity will first appear can be determined in advance of any line of rupture. There is a preliminary growth and orientation by which rows of vertically placed cells are laid down in a line across the upper central portion of the primordium. The upper cells in these rows stretch out or swell as gelatinization progresses, but the cells below the line of final separation continue to grow upward, forming a palisade-like row of elongated cells (Pl. 4, A). As noted, the cells that first rupture appear to be either swollen longitudinally or stretched. If the rupture were due to the excess growth of cells in the periphery over that of the cells within, this appearance of stretching would be a natural one; but it should be kept in mind that there has been a vertical growth of the hyphae that are being ruptured. The disorganization may thus be due to the thrust of the growing cells into those above, or to enzym action. In any event these elongating cells are the homologues of sporophores, although the ones present at this time will not bear spores. The end cells disorganize and the colloidal remains stretch out as the cavity increases in size (Pl. 5, E). The end cells slough off, new buds put forth, and new potential sporophores arise by division of cells of the enlarging dome of fertile tissue. In material grown on agar there is certaily a large amount of disorganization of tissue of the pycnidial stroma, but in nature this structure is not great, and differentiation of tissues begins very early so that the lysigenetic enlargement of the cavity is less apparent. That there is here a certain amount of cell destruction is evident from the cell remains still clinging to the inner layer of the wall of the mature pycnidium (Pls. 4, J; 6, F). So characteristic is this line of disorganized cells that our fungus was recognized in sections of oak leaves from Italy collected by Professor Massalongo 30 years ago.

DEVELOPMENT OF THE PYCNIDIUM ON STRAWBERRY

On strawberry the pycnidial primordium reaches but little size before it can be seen in sections that the cells just below the peripheral layer at the top are being oriented vertically (Pl. 4, F). There are at first not more than a half dozen rows, two or three cells in each row, in this palisade (Pl. 4, G). These appear, as it were, to be thrust against the periphery, flattening out the cells so that one would say that the wall now consists of only two or three layers of cells, disregarding the buffer tissue which is now burrowing through the host above and which takes no part in the formation of the wall. The vertically oriented hyphae now branch out and their end cells disorganize, the additional pressure due to mucilaginous disorganization tending in all cases to play a still further part in the organization of the inner-wall cells. These may in turn degenerate or, if not, they may divide tangentially or elongate peripherally so that finally the completed wall consists of five or six layers of cells which are two or three times as long as thick. Sclerosis is so long delayed that the pycnidium is capable of continued enlargement.

GROWTH OF THE BUFFER TISSUE AND FORMATION OF THE OSTIOLE

The formation of the ostiole in perithecia of a few species has been described by Füisting (*1*), De Barry (*3*), Miyabe (*4*), and others, and it has perhaps been assumed that the same principles hold in the development of the ostiole of the pycnidium. In some perithecia there is a disorganization of a strip of tissue leading from the hymenial cavity to the outside. Further enlargement of the ostiole takes place schizogenetically.

In nature the pycnidia of *Schizoparme straminea* are deep seated, and the papillate ostiole scarcely reaches the surface. The work of rupturing the epidermis and other host tissues above is performed by a sort of buffer tissue from the outer loose hyphal envelope, the "tissu fendant" of Istvanffi (*7*) or the "Aperturalgewebes" of Füisting (*1*). An opening must be made through the buffer tissue before the spores can be discharged. It will be shown that the ostiolar opening is formed first through the wall, then passage way through the buffer tissue will be made. The sharp papillate beak results from the upward growth of buds from wall cells.

Soon after the formation of the central cavity has begun, as noted above, the cells at the top of the young pycnidial knot multiply rapidly, burrow into the epidermis, and increase in size by swelling to form a cone-shaped tissue, which is very effective in lifting up, pushing aside, or breaking down the host tissues overlying the pycnidium, after which it appears above the surface as a whitish or cream-colored hood (Pl. 5, E), similar to the "tissu fendant" surmounting the pycnidium of *Coniothyrium diplodiella*. The perforation of the buffer tissue and the disorganization of the wall of the pycnidium in the preliminary stages of ostiole formation are two distinct processes, yet they are both closely associated and necessary in the development of the complete apparatus of spore discharge.

The construction of the ostiole begins at a point in the incipient inner-wall tissue above. A few cells next to the central cavity now forming (Pl. 4, B) begin to disorganize and by swelling provide a small space into which adjacent wall cells bud, turning upward (Pl. 4, E). In the meantime the buffer tissue has developed and performed its function. The disorganization of the wall cells progresses so that the ostiolar opening is

made first, after which disorganization spreads in several directions, and it is the outermost cells of the buffer tissue that are last broken down (Pl. 4, D). The swollen cells of which it is composed contain very little cytoplasm; but there are certain regions in which they show signs of degeneration, accompanied by still further swelling, so that the hood becomes split or ruptured in a very interesting fashion, the lines of cleavage radiating from the point below at which the beak of the ostiole is being formed (Pl. 5, D).

The cells of the buffer tissue still possess the power of growing, for as soon as a cavity has been formed in it by disorganization small hyphal branches bud out (Pl. 4, E). These branches are very soon entirely reabsorbed.

By the time a passage way has been opened through the buffer tissue, other short hyphae have begun to appear from the wall cells, converging to form the beak of the ostiole. The cells lining the ostiole are certainly the homologues of periphyses, but in this case they usually disorganize so that in a fully mature fruit body (Pl. 6, B) little trace of them remains. In the meantime the central cavity has become greatly enlarged, and the remnants of broken-down cells may be seen clinging to the inner wall, as noted above (Pl. 4, J). It is interesting to find that in the ascocarp of this fungus (Pl. 5, F–H) the host tissues are broken open by the same sort of buffer apparatus. This is disorganized in the same fashion and penetrated by the ostiole in like manner; the periphyses are, however, more permanent (Pl. 5, J). In the pycnidial cavity there is much more disorganization of tissue when the fungus is grown on agar media where the absence of confining host tissues may be an additional reason for the delay in the differentiation processes.

Other stages in the life history of *Schizoparme straminea* are illustrated in Plate 5. The habit of growth of pycnidia on the fruit of strawberry is brought out in Figure A, which is somewhat magnified. The buffer tissue which has broken through the epidermis of the host is the only part of the pycnidia visible. On agar media in Petri dishes the mycelium appears to break up into fan-shaped lobes, and the pycnidia form more or less in concentric circles (Fig. B). Surface views of pycnidia on agar such as are shown in Figures C and D bring out very strikingly the manner in which the buffer tissue splits open, frequently in the form of a cross.

That the buffer tissue is composed of cells originating from the upper portion of the outer wall of a pycnidium is clear from the sections shown in Figure E. It is often difficult to distinguish perithecia from pycnidia by the appearance of the buffer tissue exposed, although the ascocarps are frequently much larger than pycnidia. Figure F shows a number of perithecia as they appeared on a much decayed leaf of *Rosa* sp., bringing out well the manner of splitting the buffer tissue, which is so characteristic of the tissue above pycnidia also (Figs. C and D). Mature ascocarps collapse when dry. Sections of a young perithecium through the papillate ostiolar portion (Figs. G and H) show that the buffer tissue evidently functions in rupturing the host tissue, although it soon dries up and falls off after bursting through the epidermis (Fig. I). It is clear from Figures I and J that there are no paraphyses between the asci, although falcate periphyses line the ostiolar cavity. A mature ascocarp with fragments of buffer tissue still adhering is shown in Figure K.

We have in this species a case where the sexual and the asexual fruit bodies are very similar in several respects. The walls of both structures are composed of similar tissue, which is carbonized only at maturity. The ostiole in each form is prominent, being more or less papillate. The straw-colored buffer tissue is always present in both forms, splitting open in a characteristic fashion. The pseudoparenchymatous tissue at the center of the ascocarp breaks down, thus forming a central cavity. One need not confuse the two forms, since the perithecia react to the triple stain in a manner quite different from pycnidia.

The very striking morphological parallelism shown in pycnidia and perithecia of this species raises again the question as to what extent this really occurs among other Ascomycetes, or how much dependence can be placed on such features in the search for other spore forms in the life cycle of a given fungus. Orton (*9*) called attention especially to various cases of similarity between pycnospores or conidia and the ascospores of certain species. While many examples where there is no such resemblance between the types of spores may be pointed out, it must be admitted that it would be a very natural morphological expression of the inherent physiological or metabolic activities, based upon the constitution of the germ plasm. A feature worked out in the course of time for one spore form must of course have a basis in the apparatus of inheritance. It is surprising that these morphological parallelisms are not more frequent in the Ascomycetes, until one considers the particular environmental conditions under which each type of spore or sporocarp performs its work. The spores of *Hainesia lythri* (Desm.) v. Höhnel and *Sclerotiopsis concava* (*12*) are identical, but the sporocarps bearing them have little in common, because the latter form has taken on the overwintering function. On the other hand, the discocarp stage, Pezizella, resembles the Hainesia.

ABNORMAL ORIENTATION OF SPOROGENOUS TISSUE

It is well known that in cultures of some forms of Ascomycetes it is possible, by changing the direction of illumination, to cause pycnidia or ascocarps to be formed in an oblique or an inverted position. In such cases the structure is inverted or changed as a whole. By suitable orientation of cultures of *Schizoparme straminea* with respect to light, gravity, and aeration the fungus can be induced to build sporogenous tissue at the top of the pycnidial cavity so that the sporophores and spores point downward as they are formed, while the buffer tissue and the ostiole develop in the normal fashion, pointing upward. Figure C in Plate 4 shows a stage in the development of the pycnidium just preceding spore formation. The buffer tissue is not quite full grown, but other sections in the series show that an ostiole is just being formed through this tissue at one side of the sporogenous pulvinus so that, had this pycnidium been allowed to mature and then been cut in a plane at right angles to the one shown here, the relative positions of the sporogenous tissue, buffer apparatus, and ostiole, would be the same as shown in Pl. 6, C. In case the fertile tissue had been directly at the center above, there would have been two ostioles formed (Pl. 6, D); this is in no way associated with two locules in the early stages. What bearing can these curious inversions of the sporogenous tissue have on the question of the methods of cavity formation? This is a complicated situation where we have a central cavity formed, sporogenous tissue delimited,

buffer tissue developed through which a passageway is opened, a wall organized, an ostiolar opening through the wall portions broken down, and a beak developed. The fungus must be able to organize a number of varied localized changes and growth processes harmoniously interacting in such a way as to lead to the construction of a fruit body with certain constant diagnostic morphological features. Such a structure, the cavities or openings in which are formed purely schizogenetically, is difficult to conceive.

As *Schizoparme straminea* is found in nature there is nothing to suggest the presence of a stroma, except perhaps the buffer tissue ramifying through the host tissues and bursting forth, crowning the pycnidium. As explained, this tissue is formed after the knot of undifferentiated primordial hyphae has reached considerable size. Only one pycnidium grows from each knot of hyphae. On agar, however, several pycnida may arise from one of these masses of plectenchyma in such a way as to suggest that the line separating such pseduoparenchymatous primordia from true stromata is not to be too sharply drawn. Again, cavity formation may be started at several different points, followed by a merging of the locules, so that the cavity is at first much lobed or irregular; it finally becomes rounded out by further disorganization readjustments. A multilocular pycnidium would be a very simple step in the evolution from such a type.

As noted previously, there is only a relatively small amount of destruction of tissue during the formation of the central cavity. The ring lining the cavity (Pl. 4, J; 6, F) represents practically all of such disorganization that occurs as the fungus grows on species of Rhus or Quercus. Ostiole formation is usually not begun until the central cavity is distinguishable (Pl. 4, B and E), but no set rule seems to be followed. Ordinarily the first spores are matured before the ostiole is completed. Figure B shows a section in which the position of the ostiole is already determined by the disorganization of cells. The section (E) shows the proliferation of cells of the wall of the pycnidium and the enlargement and disorganization of cells of the buffer tissue.

Füisting (*1*) pointed out how difficult it was to find the early stages of perithecia which he was studying because there is nothing to indicate where they are forming until the Aperturalgewebes bursts through the host tissue, and by that time the perithecium is nearly mature. The same difficulty is encountered in connection with both perithecia and pycnidia of *Schizorparme straminea* as the buffer tissue is not visible until after the first steps in cavity formation have been taken.

DISCUSSION

Partly developed pycnidia of the type found in *Phyllostictina carpogena* have been considered by some authors to be merely young or undeveloped perithecia, especially when they are found in cultures. Some fungi develop vegetatively very well under artificial conditions and even reach the first stage in pycnidium formation to the extent of laying down the plectenchymatous primoridum, yet lack the particular stimulus which sets in motion the metabolic activities initiating the intermediate stages of growth which are fundamentally more complicated than the mere piling up of food in the form of undifferentiated tissue of the primordium.

Very often cell destruction is accompanied with much swelling, which may not be without effect in stimulating further action. In *Schizoparme*

straminea there is a characteristic intercalary growth of orientation before any disorganization sets in. When a weak point is once established and an initial lysigenetic cavity formed, then growth readjustments can come into play, as the results of which the structure may increase in size by the elongation of elements already present at the end of the first period of growth. Intercalary growth in the wall is stimulated, and new cells may be added to the exterior.

Bauke found that in the pycnidium of Cucurbitaria the primordium was often as large as the mature fruit body; therefore, to account for the cavity he supposed that the sporiferous layer was pressed outwardly against the intervening parenchyma so that its cells were crushed. The ultimate effect of pressure due to disorganization is certainly a factor to be recognized, but it is very doubtful whether the cells referred to are crushed to the extent claimed. Those just outside the sporiferous layer are frequently small and flat, due to division, and are not the crushed remains of the large cells once at this point in the primordium. This is quite evident, for a study of Bauke (*2, fig. 12 and 13 of Pl. II*), will show that the cells in the tissue said to be crushed must have undergone division in the process, for the ratio of the number of cells in the periphery of the primordium shown in his Figure 12 to the number in the layer beneath the sporiferious layer is as 3 to 2, while in the mature pycnidium shown in his Figure 13, this ratio is as 2 to 3. Bauke (*2*) and Baccarini (*5, 6*) both state that in old pycnidia sporophores disappear entirely, and many pycnidia have been formally described by authors as without sporophores. The writer has shown that the first hyphae to grow into the initial cavity are not necessarily spore bearing, though they are homologues of sporophores. In *Phyllostictina carpogena* the number of sporophore mother cells increases by multiplication of cells, by radial division, or by budding, while in *Sclerotiopsis concava* and *Schizoparme straminea* the sporophores increase by branching or budding of cells in the dome-shaped fertile tissue. Every cell in the thin-walled central region is potentially sporogenous. Sporophores are often sufficiently marked to be diagnostic, but that they are constant or permanent structures is not borne out by the facts learned from a study of these intermediate stages. That the sporogenous tissue by nature is fundamentally different from every other in the primordium and that, once set apart in the early ontogeny, it remains a permanent, unchanging reproductive tissue is an idea probably carried over from zoology.

Whether the contour of the sporogenous tissue is at all determined by the particular method of cavity formation may be very doubtful, but it so happens that the fertile tissue of the Phyllostictina blackrot of dewberry is strongly concave in outline; in *Sclerotiopsis concava* the incipient or potential spore tissue is convex, but by further disorganization and growth readjustments it soon becomes flattened out so that it forms an even layer extending across the base of the broad flat pycnidium; in *Schizoparme straminea* the fertile layer is sharply convex. It has been shown that in the first species the central cavity is largely lysigenetic. The cavity contains now a mass of predigested food readily available for spore formation, and it should be borne in mind that the fungus provides a large amount of food for this work when the fundamental tissue is developed so that in this pycnidium it is not necessary that more should be drawn from the vegetative hyphæ. In *Sclerotiopsis concava* the central cavity is perhaps about equally lysigenetic and schizogenetic. It is formed in much the same way as it is in the deep-seated

aecidia of the rusts [Peridermium, Adams (*11*)], where the gametes are intercalary cells below in a series of sister cells which are becoming disorganized or have been sacrificed in order to make space and to furnish food for spore production. In *Schizoparme straminea* the cavity is largely schizogenetic, although in artificial cultures on agar there is a large amount of cell destruction.

Some information has been obtained showing that the same factors, disorganization and inequalities in growth, are operative in ostiole formation. In view of the fact that the natural relative positions of the sporogenous tissue and the ostiole can be altered in a very striking manner in cultures of *Schizoparme straminea* it is much less difficult to conceive of a localized weakness in the pycnostroma determined by metabolic changes leading to disorganization which initiates cavity formation than it is to understand how the two different regions of rupture initiating the central cavity and the ostiole can be provided for so long in advance in a purely mechanical way.

In *Phyllostictina carpogena* there is usually a secondary cavity formed in the necklike extension of the immature pycnidium. This is an elliptical cavity which is finally continued downward into the pycnidium and thus becomes a part of the ostiole. Not infrequently it becomes from the first a superficial accessory pycnidial cavity lined with sporophores which cut off spores. These sporophores soon become gelatinized and an opening is formed connecting the two cavities. Sometimes the ostiolar portion is rather small and the cavity is lined with short periphyses, showing in a very striking manner that sporophores and periphyses of this species are homologous structures, or that periphyses may on occasion cut off spores.

The picture of *Schizoparme straminea* (Pl. 4, E) shows that no sooner has an opening been effected in the pycnidial wall by means of disorganization than the cells about the opening bud out or proliferate rapidly, growing into the space and turning upward, after which some of the cells at the ends of these new hyphæ in their turn become disorganized. What is it that stimulates the comparatively old wall cells to such a rapid renewal of growth? We see that there must be a change in pressure, and a new supply of food readily assimilable is furnished by the destruction of cells next to them. Even the swollen cells of the buffer tissue seemingly almost devoid of cytoplasm are still able to grow once the adjacent cells furnish a little food through disorganization.

SUMMARY

The intermediate stages in the development of the pycnidia of three species of fungi associated with fruit rots have been studied, and in each case it has been shown that the initial stage in cavity formation consists in the disorganization of cells in a certain region, accompanied by a swelling of the cell remains. The pressure thus resulting tends to enlarge the cavity, maintain a symmetrical contour, and contribute further toward cell destruction or change of form. Further enlargement of the cavity may be effected by the growth of elements already present or by the addition of new tissue.

In *Phyllostictina carpogena* the cavity is formed lysigenetically for the most part, although occasionally excessive peripheral growth plays some part in the process, especially if cavity formation begins early or before sclerosis has become far advanced.

In *Sclerotiopsis concava* the central cavity is about equally lysigenetic and schizogenetic. The swelling of certain cells of the more or less parallel hyphae composing the primordium causes a rupture along irregular lines, extending across the pycnidium, above and below, thus cutting out a strip of tissue which soon completely disorganizes. The cavity is further enlarged by the addition of new tissue at the border of the pycnidium. There is no ostiole in this pycnidium; spore discharge is effected through the bursting of the wall due to the swelling resulting from inbibition and the partial or complete destruction of certain spores at the center of the mass filling the pycnidium.

The central cavity in *Schizoparme straminea*, as it grows on strawberry, is largely schizogenetic. A ring of disorganized tissue usually lines the cavity clinging to the wall. A buffer tissue through which a passageway is opened by disorganization develops soon after the primordium has been laid down. The ostiolar cavity is formed as the result of the breaking down of the cells of the wall in the early stages; the adjacent cells bud out and hyphae grow upward, converging to form the papillate ostiolar structure. In the meantime by the rapid destruction of the buffer tissue along lines radiating from a point above the ostiole this caplike structure is split open in a characteristic fashion.

The first hyphae to grow into the central cavity as it is forming are the homologues of sporophores, but they do not necessarily produce spores, since those first appearing usually disorganize, furnishing substances which by swelling may play some part in shaping the tissues of the wall.

Food for spore production is found in the parenchyma at the center of the immature fruit body in the first two forms. The pycnidium of the third species remains plastic for a long time, so that additional food may be easily obtained from the substratum upon which the fungus is growing.

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PLATE 1

Phyllostictina carpogena Shear

A.—Section of a "pycnosclerotium"; outer cells darkly colored; central cells thin-walled.

B.—Similar structure, a little older. An open space nas been formed, and into this space the next surrounding cells have grown.

C.—Further increase in size of the central cavity. Disorganizing cell remains lie about in the cavity.

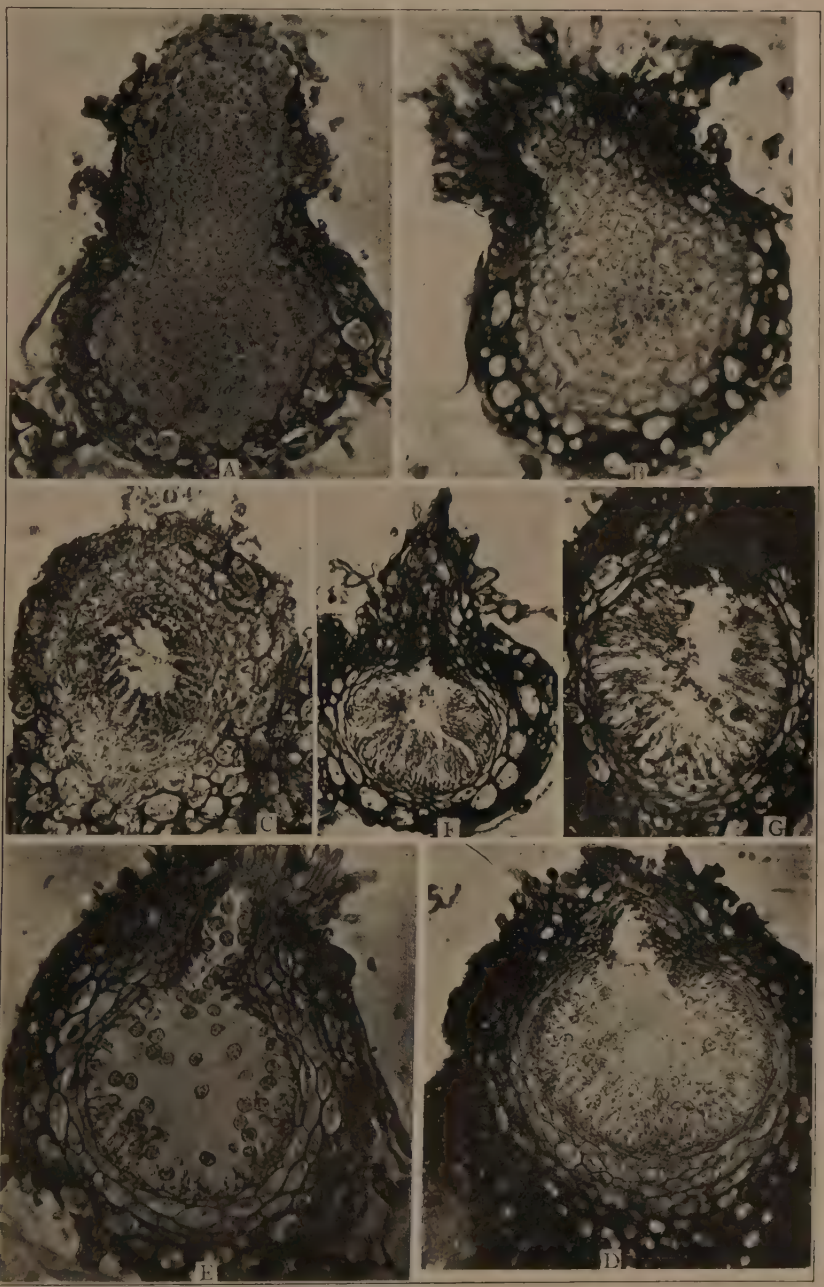
D.—Fully organized pycnidium.

E.—Mature pycnidium, showing a small elliptical ostiolar cavity.

F.—A spermogonium, showing fine, threadlike outgrowths from the inner-wall cells. They have usually disappeared by the time the first distinct spermatia are formed.

G.—An older spermogonium; spermatiophores clearly delimited; pycnospores in the cavity.

Figures B, C, D, E, and G were photographed with a 3-mm. oil-immersion lens; Figures A and F with a 4-mm. dry lens.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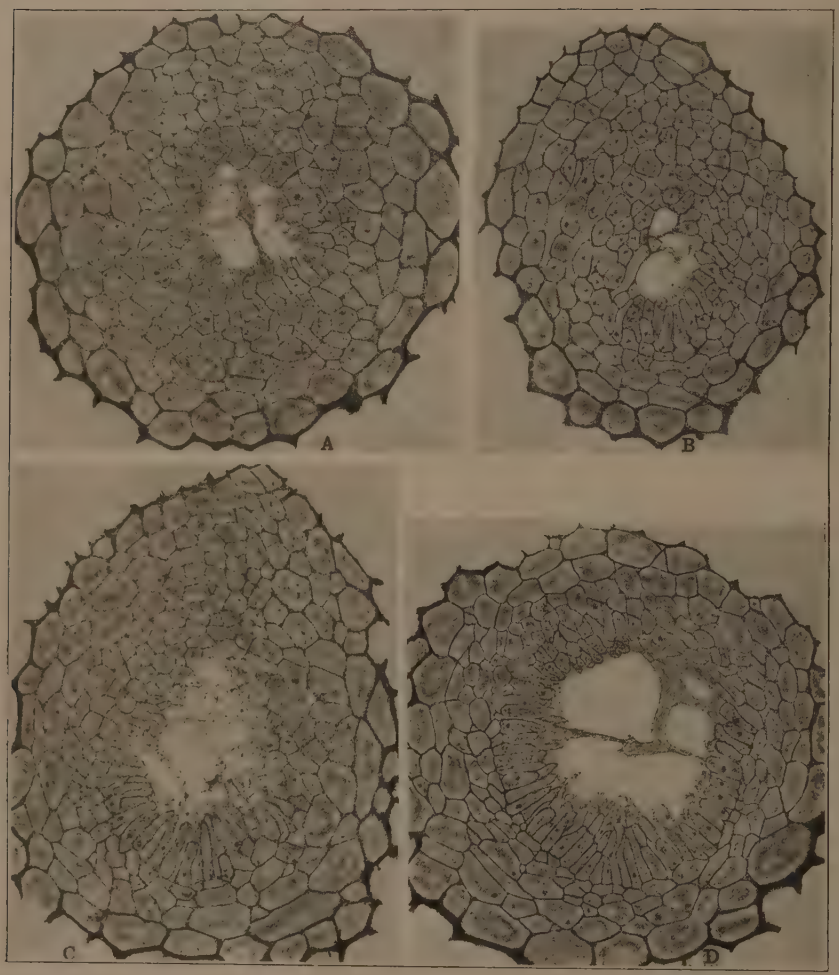
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PLATE 2

Phyllostictina carpogena Shear

A.—Primordium in which a few cells at the center have undergone disorganization; mucilaginous remains of other cells still visible; cells surrounding lower portion of the central cavity beginning to grow inward; two or three layers of "carbonized" tissue surrounding the primordium not shown.

B.—Primordium of a small pycnidium; cavity formation progressing; protosporophores growing inward; "carbonized" portions of outer wall not shown.

C.—Similar to the preceding, but protosporophores more definite.

D.—Layer of cells lining the cavity composed of protosporophores; colloidal cell remains still present in the cavity; inner wall of pycnidium becoming organized; cell divisions in various planes account for the increase in the number of cells.

Figures were drawn with the aid of camera lucida, Zeiss No. 8, ocular, 3-mm. oil-immersion lens.

PLATE 3

Sclerotiopsis concava (Desm.) Shear and Dodge

A.—Mound-shaped mass of hyphae beneath the cuticle; radial growth not evident because this is a somewhat oblique section of the primordium.

B.—Section of a primordium at a slightly later stage, showing plainly hyphae spreading outward from a broad base. The short terminal cells by slight lateral extension take part in the organization of the outer wall.

C.—Still older stage when the line of rupture is well marked. At the right can be seen a small region where the radially growing cell chains have not been ruptured. The break occurs first by the destruction of one or more of the cells of these radially oriented hyphae. The parallel arrangement of hyphal branches below the line of rupture is not the result of an upward growth after the rupture has occurred.

D.—Section of a still older pycnidium, showing quite distinctly five of six swollen cells (center) and other masses of stainable degeneration products in the cavity at the right.

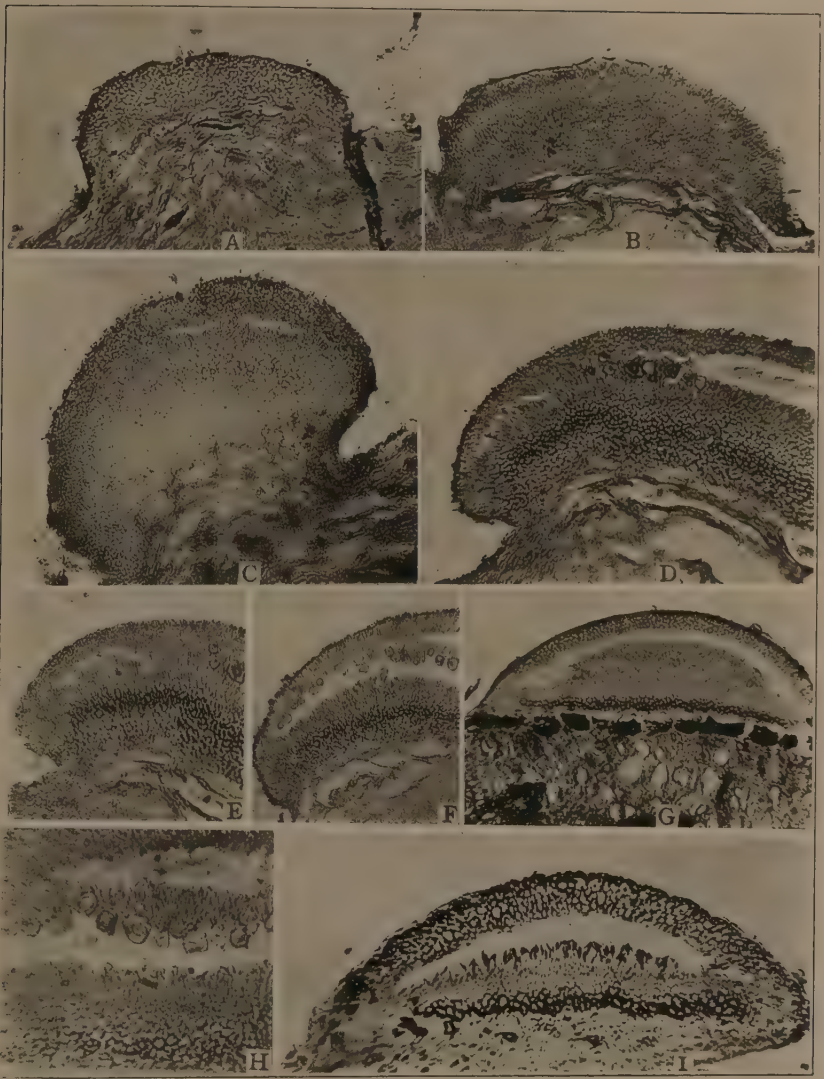
E.—Same section as the preceding except at a slightly deeper focus, showing better how the line of rupture cuts across the radially growing hyphae; degenerating cells at the right.

F.—An older pycnidium, showing many swollen cells.

G.—A pycnidium found on leaf of dewberry; cavity partly filled with masses of detached hyphal cells which originally made up the bulk of the young pycnidial "knot."

H.—More highly magnified picture of a portion of the section shown in F. The very small cells above the large ones are detached portions of the radially placed hyphae, portions of which may become the future sporophores.

I.—Section of a small pycnidium still covered by the tightly stretched cuticle. The first permanent spores formed are still attached to sporophores. There is very little of stainable cell remains in the cavity. The radial arrangement of the cells which make up the wall of the pycnidium can still be plainly recognized.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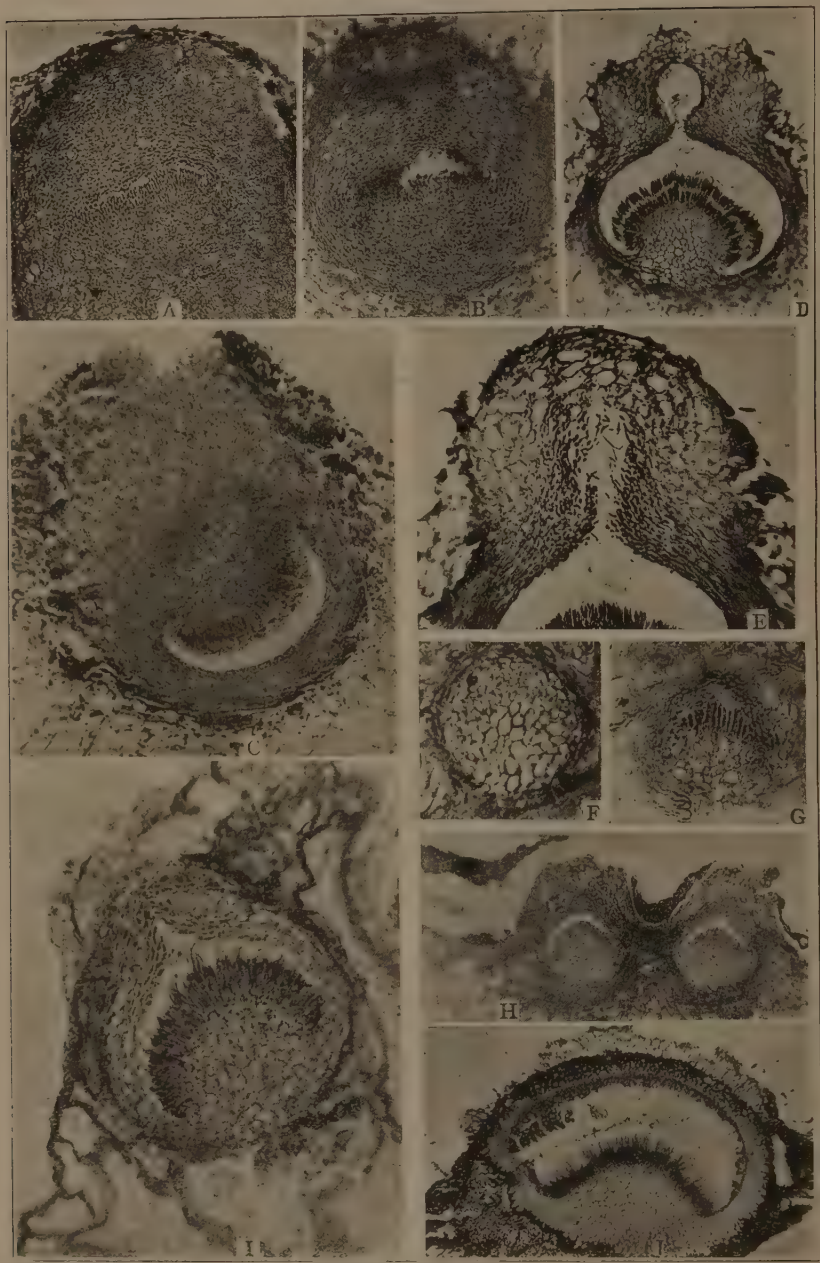
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PLATE 4[a]

Schizoparme straminea Shear

A.—Section at an early stage in the growth of a pycnidium grown on agar; cells above the line of vertically oriented hyphae disorganized, thus determining the line of rupture; no buffer tissue yet formed.

B.—A similar section at a slightly older stage; buffer tissue partly developed; location of the ostiole already determined by some disorganization of tissue; line of splitting below still being extended in both directions.

C.—Section of pycnidium grown on agar under conditions which induce the inversion of the sporogenous tissue; buffer tissue erect and normal.

D.—Section showing, like succeeding one, that the opening or rupture of the buffer tissue is caused by disorganization and not by a tearing apart due to inequalities in growth.

E.—Disorganization of cells of the hood above and proliferation of cells bordering the cavity; buds growing into the space opened by disorganization.

F.—Young pycnidial primordium on strawberry; large inner cells more or less oriented vertically. Above the center these cells are sending out short hyphal branches which are pushing into disorganizing cells in this region.

G.—Similar to the preceding; protosporophores very distinct.

H.—Section of two young pycnidia grown on strawberry. The buffer apparatus has broken through the host tissue above.

I.—Section of a pycnidium similar to the preceding; gelatinizing ends of the incipient sporophores clearly visible.

J.—Section of a pycnidium found on leaves of sumac; remains of disorganized cells lining the inner wall still attached.

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[a] Figures A, B, and H are from photographs by Marguerite Ickis.

PLATE 5[b]

A.—Pycnidia on a strawberry; only buffer tissue visible.

B.—Characteristic growth of mycelium on agar; pycnidia at first formed in concentric circles.

C.—Pycnidia from similar cultures more highly magnified, showing manner in which buffer tissue splits open frequently in the form of a cross.

D.—In older cultures many pycnidia cluster about the large one first formed at the center.

E.—Buffer tissue seen to be composed of cells originating from the upper portion of the outer wall of the pycnidium.

F.—Large perithecia from a leaf of *Rosa*, sp. They show the same peculiar splitting of the buffer tissue. Mature ascocarps always collapse when dry.

G.—Upper portion of a young perithecium, showing buffer tissue and conical ostiole; opening not yet completed.

H.—Similar to the preceding; opening in the ostiole nearly completed.

I.—Buffer tissue has dried up and broken away. Ascospores just forming; no paraphyses are ever present.

J.—Shows the falcate periphyses lining the ostiole—two branches from each cell.

K.—Mature ascocarp; remains of buffer tissue still present.

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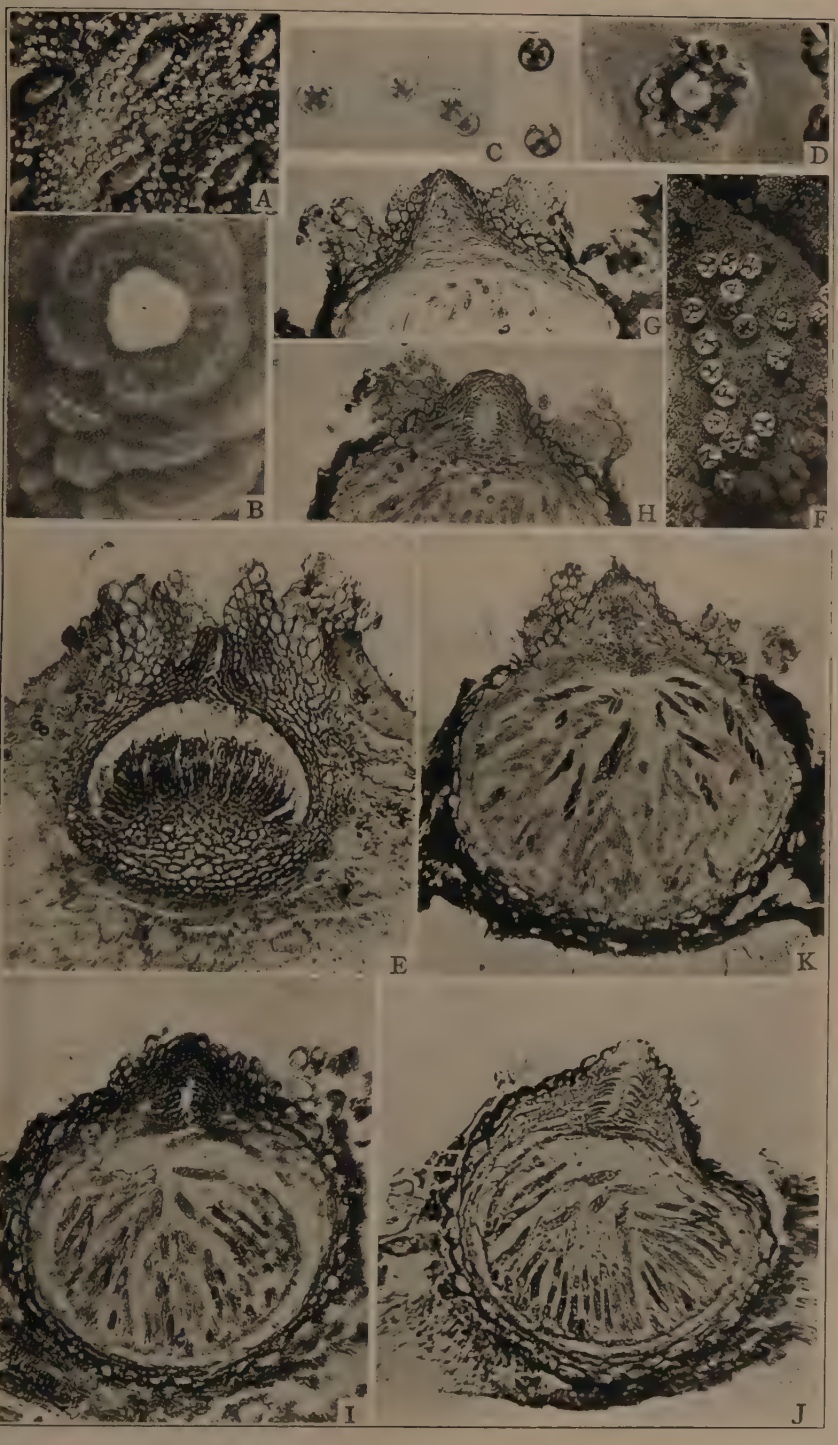
[b] Figures B and C are from photographs by Marguerite Ickis.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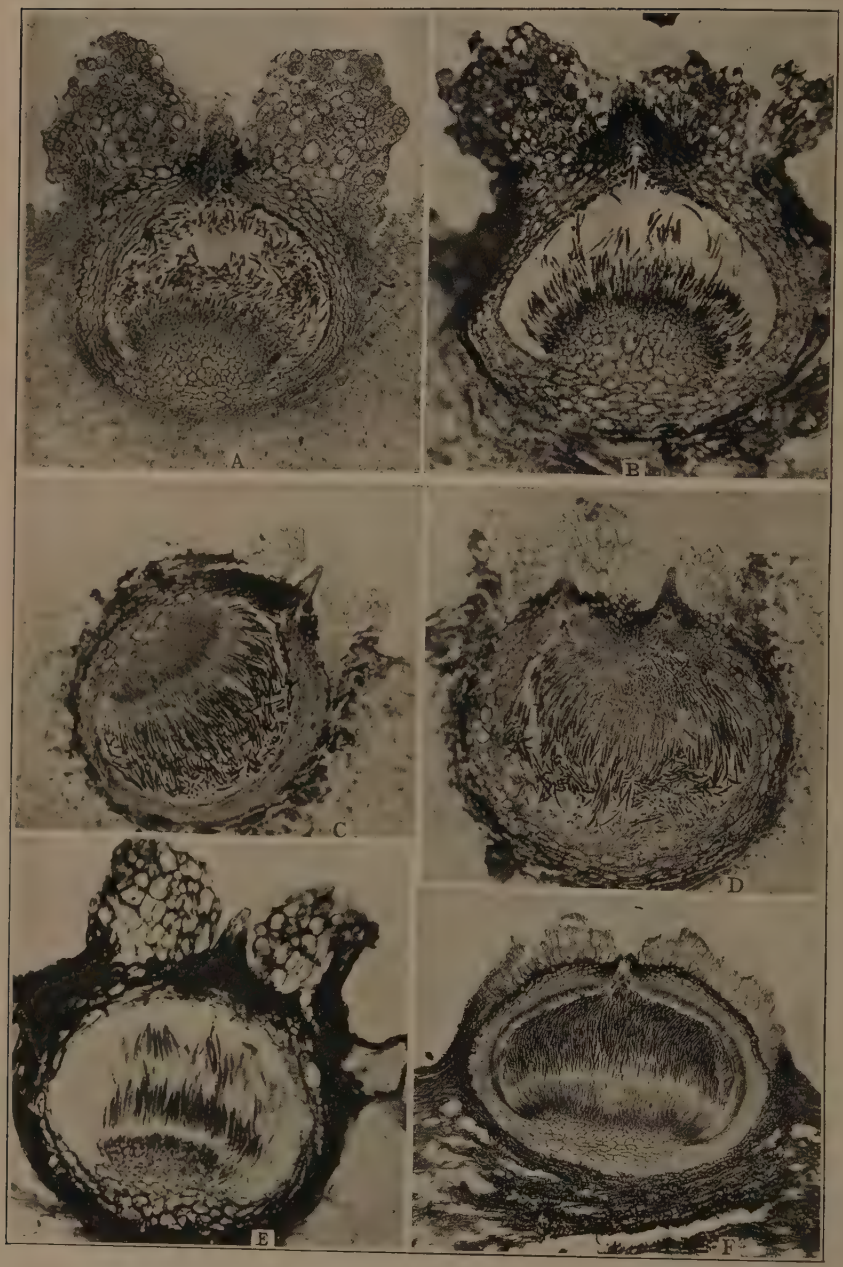
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PLATE 6[c]

Schizoparme straminea Shear

A.—Mature pycnidium grown on strawberry; buffer tissue well-developed; walls of cells surrounding the ostiole thickened and blackened; layer of stainable cell remains clinging to inner wall; sporogenous tissue below.

B.—Black conical ostiole more distinct, the only part yet "carbonized."

C.—Mature pycnidium grown on agar in such a way as to cause inversion of the sporogenous tissue; buffer apparatus penetrated by the pointed ostiole oblique to the surface of the medium.

D.—Fertile tissue completely inverted so that two ostioles are formed.

E.—Mature pycnidium grown on strawberry.

F.—Normal mature pycnidium taken from a leaf of sumac; ring of cell remains lining the central cavity.

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[c] Figure A is from a photograph by Marguerite Ickis.